

MOLECULAR GENETIC EVIDENCE FOR INTERSPECIFIC HYBRIDIZATION AMONG ENDEMIC HISPANIOLAN *BURSERIA* (BURSERACEAE)¹

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Historically, genetic introgression among species as well as hybrid origins for species of the diploid tree genus *Bursera* (Burseraceae) have been proposed based on the supposition that individuals morphologically intermediate between sympatric “parent” species must be derived from hybridization. This study reports the first molecular genetic evidence for both unidirectional and reciprocal interspecific hybridization within *Bursera*. Phylogenies of hybrids and other species in *B.* subgenus *Bursera* are reconstructed based on nuclear and chloroplast sequence data. Compelling evidence supports the hybrid origin of three endemic Hispaniolan species: *B. brunea* (*B. nashii* × *B. simaruba*), *B. gracilipes* (*B. spinescens* × *B. simaruba*), and *B. ovata* (*B. simaruba* × *B. spinescens*). Cloning studies of nuclear markers from *B. ovata* suggests that this species is an introgressed or later backcross generation hybrid and thus reproduces sexually.

Key words: *Bursera*; Burseraceae; Caribbean flora; *Commiphora*; Hispaniola; hybrid taxa; interspecific hybridization.

Bursera is a member of the frankincense and myrrh family (Burseraceae) and comprises approximately 100 species of frost-intolerant trees that range from southern Arizona, USA, to Perú, the Galápagos Islands, and the Caribbean (Bahamas plus Antilles). Species diversity and endemism for *Bursera* are centered in the Pacific drainage areas of western Mexico (60 spp., Rzedowski, 1988). Species of the genus are found in a wide variety of low elevation habitats, including deserts and rainforests but are most common in dry tropical deciduous forests (Rzedowski, 1978; Rzedowski and Kruse, 1979), where they can form a prominent component of the woody vegetation.

Since the beginning of detailed systematic work on *Bursera* in the 1930s, botanists have postulated that some species relationships within the genus may be reticulate due to interspecific hybridization. Numerous cases of introgression among species have been inferred, and the hybrid origins for seven *Bursera* species have been proposed, based on the supposition that morphologically intermediate individuals that share geographic distributions with putative parent species are derived from hybridization (Urban, 1929; Bullock, 1936, 1937, 1938; Cuatrecasas, 1957; McVaugh and Rzedowski, 1965; Rzedowski, 1968; Wiggins and Porter, 1971; Toledo-Manzur, 1982; Rzedowski and Ortiz, 1988; Rzedowski and Guevara-Féfer, 1992). Although hybridization has been postulated to occur equally within the two subgenera of *Bursera*, *B.* subg. *Elaphrium* and *B.* subg. *Bursera*, no hybridization between subgenera has been reported even though they have sympatric ranges. Purported hybrid species are presumed to be diploid because none of the taxa examined to date has morphological features associated with polyploid taxa, such as enlarged pollen grains, leaves, and flowers (Stebbins, 1950). Authors of the most recent taxonomic synopsis of *Bursera*, which in-

cludes only those species from western Mexico, accepted that hybridization occurred but did not comment on how it may have influenced the evolution of the genus (McVaugh and Rzedowski, 1965, p. 322). They prescribed additional field studies of hybrid taxa in order to “profitably speculate about the extent of hybridization and introgression” within the genus *Bursera*.

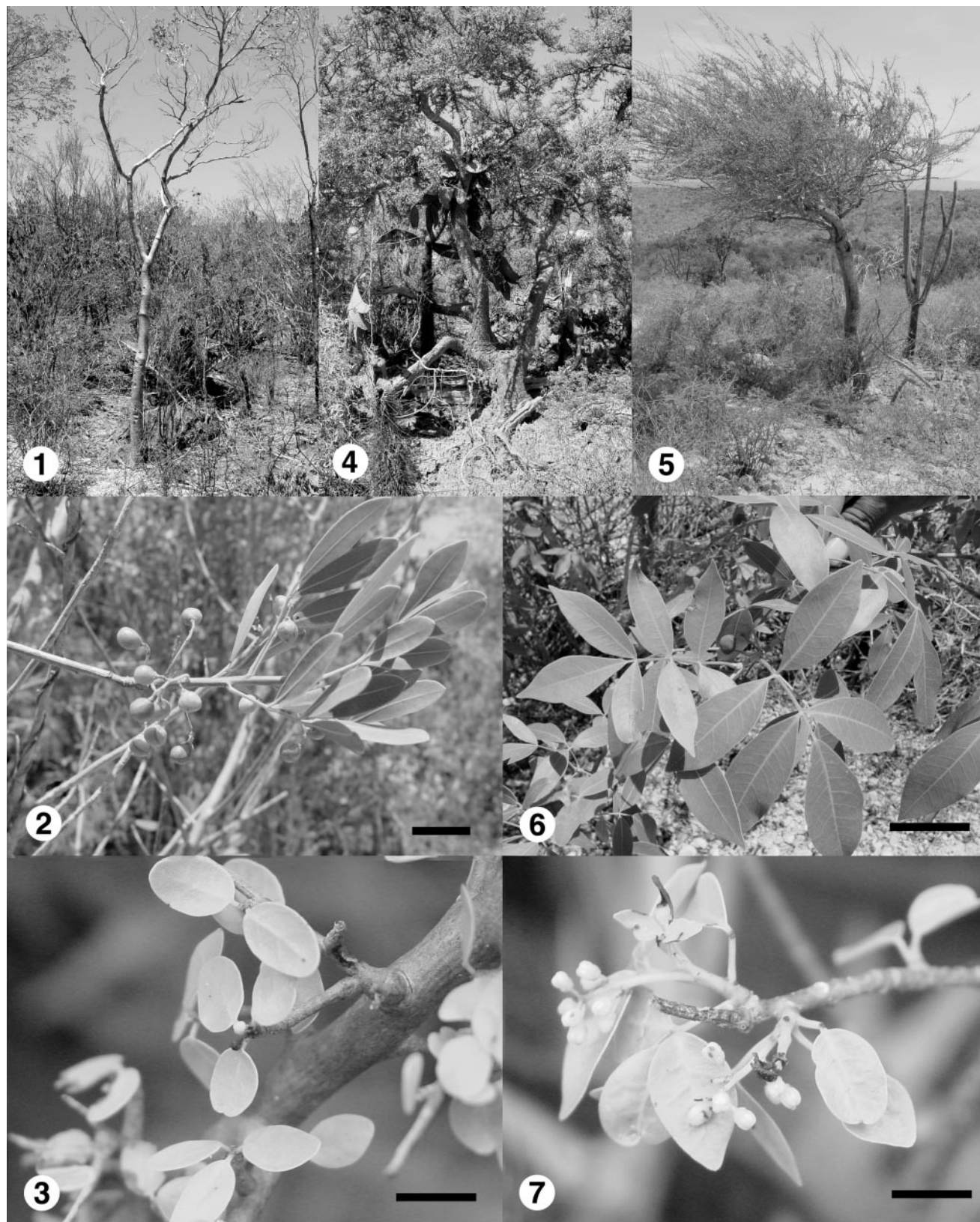
Although Mexican *Bursera* species have been the focus of molecular phylogenetic work (Becerra, 1997, 2003; Becerra and Venable, 1999), no published studies to date have used DNA markers to test putative hybrid species or to examine whether reticulate evolution is a component of *Bursera*’s genetic history. The only detailed examination of a putative interspecific hybrid in *Bursera* focused on the reproductive biology of *B. medranoana* (*B.* subg. *Bursera*; Cortés-Palomec, 1998) but did not test the hypothesized parentage (*B. morelensis* × *B. schlechtendalii*) or establish the directionality of the hybridization event. Cortés-Palomec’s findings suggest that the role of *Bursera* hybrids in species formation or in the introgression of genes among existing species may be extremely limited because *B. medranoana* lacks viable pollen and reproduces asexually through apomixis.

During field collection of *Bursera* species on the Caribbean island of Hispaniola for our molecular systematic investigations of the Burseraceae and the genus sister to *Bursera*, *Commiphora*, we found three different putative interspecific hybrids involving members of *B.* subg. *Bursera*. The first two hybrids appeared to share *B. simaruba* as a parent. This species is common throughout the Caribbean and (sub)tropical regions of North, Central, and northern South America (Francis, 1990). We hypothesized that the other parents of these two hybrids were species endemic to Hispaniola, *B. nashii* (Figs. 1, 2) and *B. spinescens* (Figs. 3, 4). We later determined that the two hybrids corresponded to two named endemic species, *B. brunea* and *B. gracilipes*, respectively. Interestingly, Urban (1929, p. 59) noted that E. L. Ekman, who transferred *Spondias brunea* to *Bursera*, suspected that this species was a hybrid between *B. simaruba* and *B. nashii*. We inferred that a third endemic Hispaniolan species, *B. ovata* (Fig. 5), was also a hybrid based on its morphological similarity to *B. gracilipes*, the hypothesized hybrid of *B. spinescens* and *B. simaruba*.

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Figs. 1–7. *Bursera* species and interspecific hybrids endemic to Hispaniola. 1. *Bursera nashii*, view of habit. 2. *Bursera nashii*, leaves and infructescences. Bar = 2 cm. 3. *Bursera spinescens*, leaves and sessile flowers. Bar = 1 cm. 4. *Bursera spinescens*, view of habit. 5. *Bursera ovata*, view of habit. 6. *Bursera brunea*, leaves and fruit. Bar = 5 cm. 7. *Bursera gracilipes*, leaves and inflorescences. Bar = 1 cm.

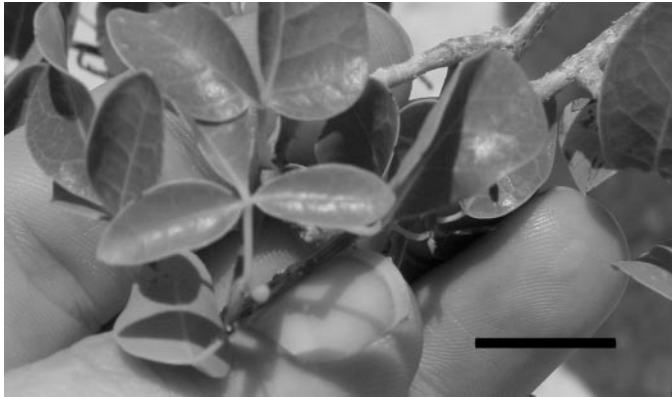


Fig. 8. *Bursera ovata*, leaves and single flower. Bar = 1 cm. Note similarity to *B. gracilipes* (Fig. 7).

The putative hybrid, *Bursera brunea* (Fig. 6), was discovered within sight of its likely parent species, *B. simaruba* and *B. nashii*, and was morphologically intermediate between the two. Only a single individual was found. The leaf size and leaflet shape were similar to *B. simaruba*, but the coriaceous texture and thickness of the leaflets was closer to that of *B. nashii*. We found two individuals of the putative hybrid, *B. gracilipes* (Fig. 7). These looked mostly like *B. spinescens* in their spinescent habit but had larger simple leaves than *B. spinescens* and pinnately compound leaves atypical of that species. The racemose inflorescences of *B. gracilipes* were similar to those of *B. simaruba* as opposed to those of *B. spinescens*, which are sessile and have 1–2 flowers. The partially spinescent habit and striking similarity of the leaves of *B. ovata* (Fig. 8) to those of *B. gracilipes* led us to speculate that *B. ovata* was also a hybrid between *B. simaruba* and *B. spinescens*, although inflorescence structure was clearly different between the two species.

Interspecific hybrids are most commonly identified today by recovering different species relationships in nuclear and plastid based phylogenies, which denote different parental contributions to the hybrid genome (cf. *Quercus*, Whittemore and Schaal, 1991; *Populus*, Keim et al., 1989; *Helianthus*, Rieseberg, 1990; *Penstemon*, Wolfe and Elisens, 1993; *Rhododendron*, Milne et al., 1999). Nuclear alleles are biparentally inherited, whereas plastids are typically inherited from the maternal parent in angiosperms (Corriveau and Coleman, 1988; Hagemann and Schröder, 1989; Harris and Ingram, 1991). To test the parentage of these putative *Bursera* hybrids, DNA sequence data from the nuclear and chloroplast genomes were collected from the putative hybrids and all other Hispaniolan species, as well as other Caribbean and continental *Bursera* species in *B.* subg. *Bursera*. Multiple independent phylogenies of *B.* subg. *Bursera*, including the hybrid individuals, were constructed using these sequence data sets under the parsimony criterion.

Selection of molecular markers—Our pilot sequencing studies revealed very low levels of sequence variation in the nuclear and chloroplast genome of *Bursera* species. This indicated that only sequence data from rapidly evolving DNA regions would be useful for elucidating species relationships. From the nuclear genome, the external transcribed spacer (ETS) and the fourth intron of the phosphoenolpyruvate carboxylase gene (PEPC) were selected for the phylogenetic

study. The ETS is part of the 3' end of the intergenic spacer located between the 26S and 18S genes of the nuclear ribosomal (nrDNA) repeat region; it may participate in the post-transcriptional processing and maturation of the ribosomal subunits (Musters et al., 1990; Hitchen et al., 1997). This region has provided informative variation at the species level for various groups of the Asteraceae and allied families (Baldwin and Markos, 1998; Linder et al., 2000; Markos and Baldwin, 2001), Rosaceae (Vanden Heuvel and Linder, 1999), Fabaceae (Bena et al., 1998), and Myrtaceae (Wright et al., 2001). Phylogenies derived from nuclear markers not associated with the nrDNA repeat region can provide an additional test of the concordance between species phylogenies and nuclear gene phylogenies (Sang, 2002). Phosphoenolpyruvate carboxylase catalyzes the synthesis of oxaloacetate from phosphoenolpyruvate and bicarbonate. It is ubiquitous in plants, and in C₄ or crassulacean acid metabolism (CAM) plants, it replaces ribulose biphosphate carboxylase as the enzyme responsible for the initial capture of dissolved carbon dioxide from the cytosol (Latzko and Kelly, 1983; Westhoff and Gowik, 2004). It is part of a low copy gene family, and cDNA studies of phosphoenolpyruvate for C₃ species suggest these copies are orthologous, whereas those for C₄ and CAM species are not (Gehrig et al., 1998). Studies of the fourth intron of PEPC within the Moringaceae (Olson, 2002) have shown that only one copy is directly amplified from DNA extracts and that it can provide levels of sequence variation appropriate for infrageneric studies.

From the chloroplast genome, the *rps16* intron and the *psbA-trnH* spacer were selected for the phylogenetic study from 10 other genes, intergenic spacers, and introns tested: *rpl16* intron, *trnL* intron, *trnL-trnF* spacer, *trnT-trnL* spacer, *trnS-trnG* spacer, *psbB-psbF* spacer, *atpB-rbcL* spacer, *matK*, the 5' and 3' *trnK-matK* spacers. The *rps16* intron is easily amplified using primers anchored in the highly conserved regions of the flanking exons, and, as a chloroplast group II intron, it is likely to have been inserted into its current position only once (Kelchner, 2002). This intron has been used in molecular phylogenetic studies at the infrafamilial level and above (Sileneae, Oxelman et al., 1997; Rubioideae, Andersson and Rova, 1999) but has been shown to provide some infrageneric sequence variation as well (*Margaritopsis*, Andersson, 2001; *Alectryon*, Edwards and Gadek, 2001; *Silene*, Popp and Oxelman, 2001). The *psbA-trnH* intergenic spacer is located between the photosystem II gene, *psbA*, and a component gene for the tRNA-histidine complex, *trnH^{GUU}* (Aldrich et al., 1988). The gene *psbA* is adjacent to the right-hand copy of the chloroplast inverted repeat, and genetic mapping studies have shown this region can undergo structural mutation (Palmer, 1985). Sequencing studies of the *psbA-trnH* spacer (*Sonchus*, Kim et al., 1999; *Aconitum*, Utelli et al., 2000) have shown that this region can provide informative base pair and length variation among species.

MATERIALS AND METHODS

Taxon sampling and DNA extraction—The 17 *Bursera* species and hybrids sampled are listed in Appendix 1 (see Supplemental Data accompanying the online version of this article). Two accessions of *B. simaruba* were included from the Dominican Republic and Florida. The outgroup species, *B. tecomaca* of *B.* subg. *Elaphrium*, was selected on the basis of existing molecular phylogenetic evidence (Becerra, 1997; Weeks, 2003). Whole genomic DNA was extracted following the protocol of Doyle and Doyle (1987) using a modified extraction buffer including 1% (w : v) polyvinylpyrrolidone and 0.2% (v : v)

beta-mercaptoethanol. DNA extracts were cleaned further using cesium gradient centrifugation (Palmer, 1986) at 20°C at 60 000 rpm for 10 h.

Amplifying nuclear markers—We sequenced a 330-base pair (bp) fragment from the 3' end of the ETS upstream from the 18S gene using Bur-ETS1F (5' TTC GGT ATC CTG TGT TGC TTA C 3') and a previously published 18S primer (Baldwin and Markos, 1998). The polymerase chain reaction (PCR) volumes of 25 µL included: 10–100 ng of template DNA, 2× PCR buffer (Epicentre, Madison, Wisconsin, USA), 1 mmol/L MgCl₂, 400 µmol/L each dNTP, 0.4 mmol/L of each primer, and 1 unit of *Taq* polymerase. This region was amplified using an initial denaturation of 95°C (5 min; after which *Taq* was added to each reaction), 35 cycles of denaturation at 94°C (3 min), 56°C (1 min), 72°C (1 min 20 s + 3 s/cycle), and a final extension period of 72°C (7 min). The PCR products of ETS were cloned for each nonhybrid and hybrid taxon sampled (TOPO-TA Cloning; Invitrogen, Carlsbad, California, USA) using one-third the recommended reaction volumes. Three or more positively transformed colonies were sequenced bidirectionally for each accession.

The PEPC intron was amplified using the primer pair PPCX4F and PPCX5R (Olson, 2002). The PCR reaction volumes of 25 µL included: 10–100 ng of template DNA, 1× Triton-X PCR buffer, 2 mmol/L MgCl₂, 400 µmol/L each dNTP, 0.4 mmol/L each primer, and 1 unit of *Taq* polymerase. This region was amplified using the temperature cycling protocol as outlined in Olson (2002). For each putative hybrid taxon, PEPC intron PCR products were cloned and three or more positively transformed colonies were sequenced bidirectionally. Two sizes of PCR product were detected by gel electrophoresis for *B. microphylla*. One fragment was similar in length to those from other *Bursera* species sampled and one fragment was shorter (ca. 500 bp). These two copies were cloned and sequenced. The longer copy, which consisted of one type within *B. microphylla*, was easily alignable to the rest of the data matrix, whereas the shorter copy was unalignable with our other sequences from *Bursera*, although it did partially match other PEPC coding regions published in GenBank. At a higher annealing temperature of 59°C, only the longer fragment was amplified. We concluded that the shorter fragment was not the functional fourth intron of the PEPC gene complex. Instead, it may be the first, second, or third PEPC intron or be a part of a nonfunctional PEPC gene complex that was amplified due to similarity of primer annealing sites.

Amplifying chloroplast markers—The *rps16* intron and the *psbA-trnH* intergenic spacer were amplified using previously published primers (Oxelman et al., 1997; Sang et al., 1997). The PCR protocols were identical to those listed for the PEPC intron. The two chloroplast regions were amplified using an initial denaturation step of 95°C (5 min; after which 1 unit *Taq* polymerase was added to each reaction), and 35 cycles of 94°C (3 min), 50°C for *psbA-trnH* (1 min) or 57°C for *rps16* (1 min), 72°C (1 min 20 s + 3 s/cycle) and a final extension period of 72°C (7 min). Although chloroplast PCR products from all putatively hybrid taxa could be amplified and sequenced directly without cloning, PCR products of chloroplast regions for *B. brunea* and *B. gracilipes* were cloned to test whether only one of two (or more) divergent haplotypes was being amplified due to PCR bias. Two or three clones from each of the two species were sequenced bidirectionally.

DNA sequencing—The PCR products were verified prior to sequencing using agarose gel electrophoresis and were cleaned using Qiaquick columns (Qiagen, Valencia, California, USA) or Centri-Sep columns (Princeton Separation, Aldelphia, New Jersey, USA) packed with G-50 Sephadex (Amersham Biosciences, Uppsala, Sweden). Each region was prepared for bidirectional sequencing following a cycle sequencing protocol including the original amplification primers, Big Dye fluorescent dye-terminator reagent mix (Applied Biosystems, Norwalk, Connecticut, USA), and 20–40 ng of template DNA. The PCR cycling protocol used a temperature ramping rate of 1°C/s and included an initial denaturation of 96°C (1 min) followed by 25 cycles of 96°C (10 s), 50°C (5 s), 60°C (4 min) with a final extension period of 72°C (7 min). Samples were cleaned with Centri-Sep columns and sequenced either at the Institute of Cellular and Molecular Biology DNA Core Facility, The Univer-

sity of Texas at Austin using an ABI Prism 3700 automated sequencer (Applied Biosystems, Foster City, California, USA), or by the authors using a BaseStation automated sequencer (MJ GeneWorks, San Francisco, California, USA).

Sequence alignment and analysis—Sequence strands were assembled and edited in Sequencher (Gene Codes, 1995). The sequences were aligned automatically in ClustalX (Thompson et al., 1997) and then adjusted manually in SeqApp (Gilbert, 1992) and MacClade (Maddison and Maddison, 2000). To search the maximum amount of potential tree space within a reasonable computational time, 1000 random addition replicates of the data were searched holding and swapping on 10 trees at each step, greater than 1 step in length, using tree-bisection-reconnection (TBR) branch swapping with Multrees in effect in PAUP* version 4 beta 10 (Swofford, 2002). These trees were saved and were then used as the basis for another round of TBR swapping up to an arbitrary maximum of 20 000 trees. From these 20 000 trees (or fewer), a strict consensus was generated. Bootstrap support for clades was assessed using 1000 pseudoreplicates of the data within PAUP*.

The ETS clone data set was reduced for nonhybrid taxa to compare and combine it with the PEPC intron data set and to reduce homoplasy due to the presence of multiple, orthologous copies. The full ETS data set, including all clones, was reduced to a single ETS clone for each nonhybrid taxon three times, while still retaining all clones from putatively hybrid taxa, and was analyzed using the parsimony method as outlined previously. Clones from each species were numbered based on the order by which they were amplified. The three reduced data sets consisted of (1) the lowest numbered clones, (2) the intermediate numbered clones, (3) the highest numbered clones from each of the nonhybrid taxa plus all the clones from putatively hybrid taxa. The number of trees generated from these three data sets varied widely (44, 352, 704 trees), although the topologies of their strict consensus trees did not disagree with the species relationships as outlined by the phylogeny of all ETS clones. As an alternative solution, consensus sequences were generated for each nonhybrid taxon by condensing into ambiguities (= polymorphisms) all base-pair differences among clones. This data set was chosen for further analysis because it retained all base-pair variations present within taxa and did not inflate the number of autapomorphic characters.

Congruence among data sets within each genome was determined by the incongruence length difference test (partition homogeneity test option in PAUP*) using 100 bi-partitions of the data. The condensed ETS and PEPC intron data sets were combined by concatenating sequence data of nonhybrid taxa and by inserting missing data in hybrid clone sequences such that all hybrid clones were included as individual terminal taxa in the expanded aligned data set. Data sets of the *rps16* intron and *psbA-trnH* spacer were combined similarly.

RESULTS

All directly sequenced data are available in GenBank (accession numbers AY309280–AY309409, URL: <http://www.ncbi.nlm.nih.gov/Genbank>) and alignments of all five individual data sets are available on TreeBase (accession numbers M1699–M1703, URL: <http://www.treebase.org>). Results from parsimony analyses of all individual data sets are listed in Table 1.

Nuclear markers—Alignment of sequence data for ETS and PEPC intron was unambiguous. Parsimony analysis of the ETS data set containing all clones from nonhybrid and hybrid taxa yielded 20 000 most parsimonious trees of 148 steps. Of 370 characters, 270 were constant, 25 were autapomorphic, and 75 were informative. For nonhybrid taxa, multiple copies were either invariant or had up to eight base-pair differences. The multiple copies coalesced as monophyletic groups or were reduced to a polytomy with other closely related species (Fig. 9). For putative hybrid taxa, ETS copies were divided among

TABLE 1. Summary of results from parsimony analyses of all data sets, a maximum of 20 000 most parsimonious trees was saved during each analysis.

Data set	Aligned, trimmed length (bp)	No. parsimony informative characters (%)	No. most parsimonious trees	Length of most parsimonious trees	CI	RI	RC
ETS all clones	370	75 (20.3)	20 000	148	0.770	0.948	0.730
ETS condensed	370	48 (13.0)	264	113	0.796	0.900	0.716
PEPC intron	654	54 (8.3)	3	132	0.924	0.962	0.889
<i>rps16</i> intron	842	23 (2.7)	4	52	0.962	0.978	0.940
<i>psbA-trnH</i> spacer	557	27 (4.8)	16	56	0.923	0.967	0.893

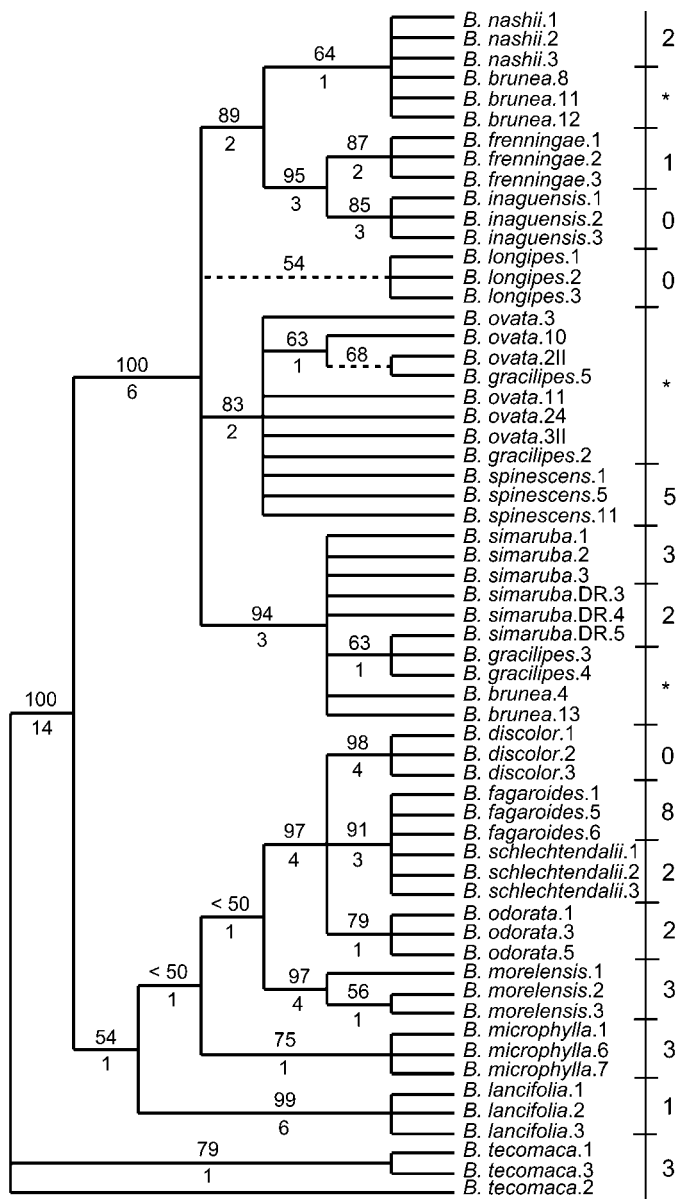


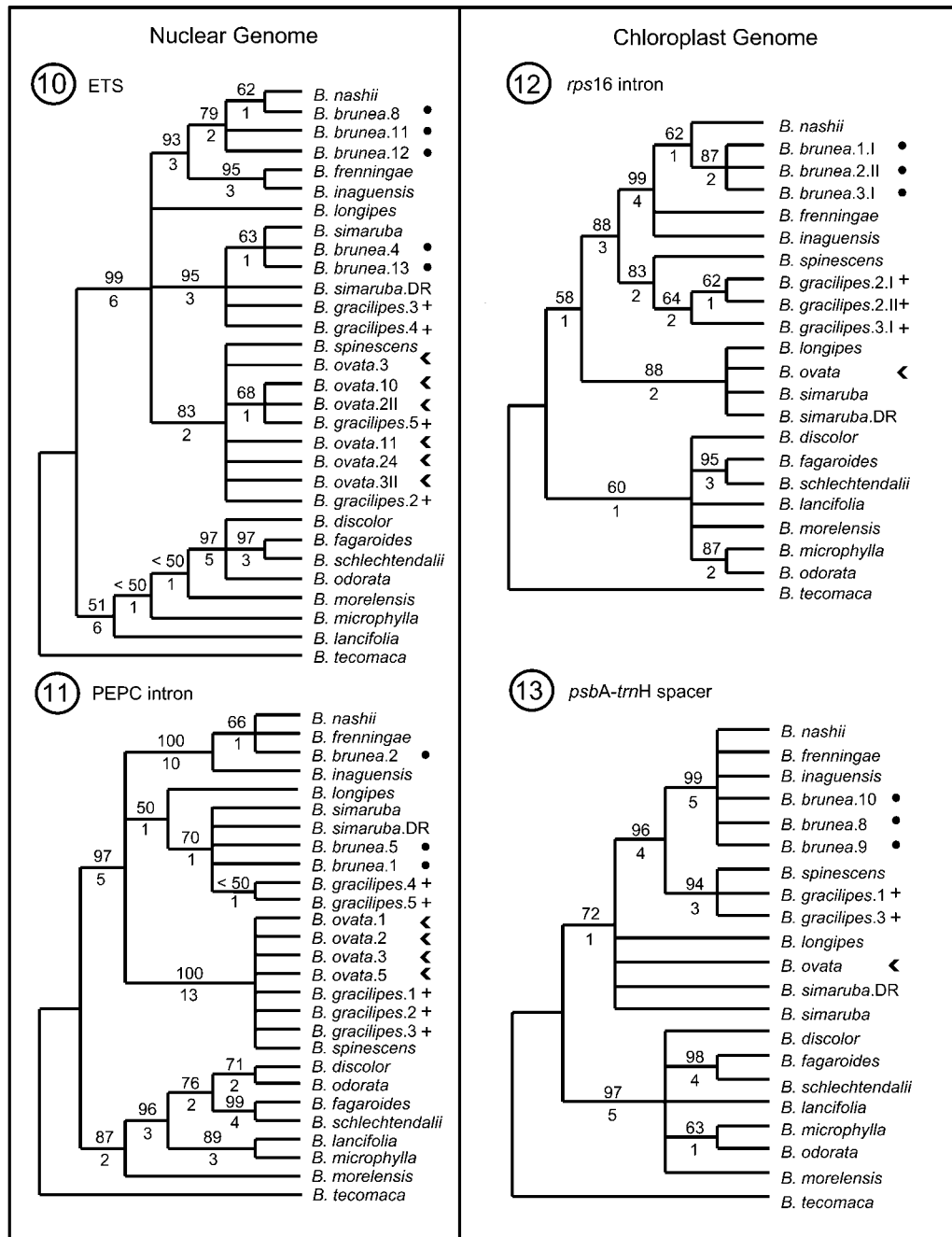
Fig. 9. Strict consensus phylogenies from the analysis of all ETS clone data, bootstrap values (from 1000 pseudoreplicates) listed above branches, decay indices below. Dashed lines represent groupings present only in the bootstrap 50% majority rule tree. Number of base-pair differences between coalescent ETS clones of each nonhybrid taxon listed vertically on right hand side, numbers of base-pair differences between noncoalescent ETS clones from putative interspecific hybrids *Bursera brunea*, *B. gracilipes*, and *B. ovata* (denoted by *) are detailed in the body of the text. *Bursera simaruba* DR is the accession from the Dominican Republic.

distantly related clades that contained their hypothesized parent species. The ETS clones of *Bursera gracilipes* were divided between the *B. simaruba* clade (eight pairwise site differences among coalescent clones of the same hybrid taxon) and the clade containing a polytomy of *B. spinescens* and *B. ovata* ETS clones (no sites). One ETS clone of *B. gracilipes* was more closely related to two clones of *B. ovata*. The ETS clones of *B. brunea* were divided between the *B. simaruba* (one site) and the *B. nashii* clade (none to one site). Clones of *B. ovata* were nested with those of *B. spinescens* and the putative hybrid *B. brunea* (1–9 sites). Parsimony analysis of the condensed ETS data set yielded 264 trees of 113 steps (Fig. 10). Of 370 characters, 291 were constant, 31 were autapomorphic, and 48 were informative.

Parsimony analysis of PEPC intron sequences, including those cloned copies from putatively hybrid taxa, yielded three trees of 132 steps (Fig. 11). Of 654 characters in the aligned matrix, 536 were constant, 64 were autapomorphic, and 54 were informative. Clones of *Bursera brunea* were divided between the *B. simaruba* clade (two sites) and in the *B. nashii*–*B. frenningae* clade (one clone). The clones of *B. ovata* were limited to the *B. simaruba* clade (none to one site). Clones of *B. gracilipes* were divided between the *B. simaruba* clade (no sites) and the clade containing the polytomy of *B. spinescens* and *B. ovata* (no sites).

Chloroplast markers—Parsimony analysis of *rps16* intron sequence data, including those cloned copies from the putative interspecific hybrids, yielded four trees of 52 steps (Fig. 12). Of 842 characters in the aligned matrix, 793 were constant, 26 were autapomorphic, and 23 were informative. Clones of *Bursera brunea* clustered only in the *B. nashii* clade (none to two sites), and clones of *B. gracilipes* clustered only in the *B. spinescens* clade (none to three sites). *Bursera ovata*, which was represented by an uncloned sequence, nested in the unresolved *B. simaruba*–*B. longipes* clade. Parsimony analysis of the *psbA-trnH* intergenic spacer region yielded 16 trees of 56 steps (Fig. 13). Of 557 characters in the aligned matrix, 510 were constant, 20 were autapomorphic, and 27 were informative. Clones of *B. gracilipes* clustered in the *B. spinescens* clade (one site) and clones of *B. brunea* clustered in the unresolved clade (no sites) containing *B. nashii*, *B. frenningae*, and *B. inaguensis*. The uncloned sequence of *B. ovata* was reduced to a polytomy with *B. simaruba* and *B. longipes*.

Combined genome data sets—Nonsignificant results from incongruence length difference tests of the nuclear (1024 bp) and chloroplast (1399 bp) data sets established that each was internally congruent ($P = 0.57, 0.52$, respectively). Parsimony and bootstrap analyses of the combined nuclear and combined



Figs. 10–13. Strict consensus phylogenies of Hispaniolan *Bursera* based on DNA sequence data from the nuclear (Figs. 10, 11) and chloroplast (Figs. 12, 13) genome. **10.** The condensed ETS data set. **11.** The PEPC intron data set. **12.** The *rps16* intron data set. **13.** The *psbA-trnH* intergenic spacer data set. Clade support values from bootstrap analyses are listed above branches, decay indices are listed below. Noncoalescent clones from putative interspecific hybrids are denoted by symbols: *B. brunea* (bullet), *B. gracilipes* (cross), and *B. ovata* (chevron). *B. simaruba* DR is the accession from the Dominican Republic.

chloroplast data sets (figures not shown) gave results consistent with those from the individual data sets.

DISCUSSION

Placement of nuclear clones from the two putative *Bursera simaruba* hybrids, *B. brunea* and *B. gracilipes*, is as expected for recently derived interspecific hybrids (Rieseberg, 1990, 1996). The presence of a single chloroplast haplotype in each putative *Bursera* hybrid indicates that the inheritance of the

chloroplast genome is uniparental but does not prove that inheritance is maternal in all cases. We assume that inheritance of plastids in *Bursera* is maternal, as is the case in most (73%) angiosperms (Harris and Ingram, 1991). *Bursera spinescens* is indicated as the maternal parent of *B. gracilipes*, whereas *B. nashii* is indicated as the maternal parent of *B. brunea*. *Bursera gracilipes* and *B. brunea* also combine the nuclear alleles of their maternal parents and their other hypothesized parent species, *B. simaruba*, which demonstrates the genetic additivity of their hybrid nuclear genomes. Thus, we can infer from

TABLE 2. Exhaustive list of *Bursera* species distributed in the Caribbean (Bahamas plus Antilles). IUCN Red List taxon noted by asterisk (*).

Species	Distribution
<i>B. angustata</i> Griseb.	Cuba
<i>B. aromatica</i> Proctor*	Jamaica
<i>B. brunea</i> Urb. & Ekman	Hispaniola: Haiti, Dominican Republic
<i>B. fremingae</i> Correll	Bahamas: Great Exhuma, Cat Island, Long Island
<i>B. glauca</i> Griseb.	Cuba
<i>B. gracilipes</i> Urb. & Ekman	Hispaniola: Haiti, Dominican Republic
<i>B. graveolens</i> (H.B.K.) Triana & Planch	Cuba, Mexico, Central and N. South America
<i>B. hollickii</i> Fawcett & Rendle	Jamaica
<i>B. inaguensis</i> Britton	Bahamas: Little Inagua
<i>B. lunanii</i> (Spreng.) C.D. Adams & Dandy ex Proctor	Jamaica
<i>B. nashii</i> Urb.	Hispaniola: Haiti, Dominican Republic
<i>B. ovata</i> Urb. & Ekman	Hispaniola: Dominican Republic
<i>B. shaferi</i> Urb.	Cuba
<i>B. simaruba</i> (L.) Sarg.	USA, Caribbean, Mexico, Central and N. South America
<i>B. simplicifolia</i> DC.	Jamaica
<i>B. spinescens</i> (Urb.) Urb. & Ekman	Hispaniola: Haiti, Dominican Republic
<i>B. tatamaco</i> Triana & Planch.	Grenada

these results that *B. gracilipes* equals *B. spinescens* (♀) × *B. simaruba* (♂) and that *B. brunea* equals *B. nashii* (♀) × *B. simaruba* (♂).

It is impossible to determine from the phylogenies of Hispaniolan *Bursera* whether *B. gracilipes* and *B. brunea* represent F1 generation hybrids or later backcrosses because of the erratic behavior of concerted evolution. Studies of molecular evolution in hybrid as well as nonhybrid plants have revealed that paralogous copies of the nrDNA repeat may be maintained in ancient lineages despite sexual recombination and cladogenesis (cf., Winteraceae, Suh et al., 1993; *Paeonia*, Sang et al., 1995; *Gossypium*, Wendel et al., 1995; *Pinus*, Gernandt et al., 2001; *Quercus*, Muir et al., 2001). Investigation of the reproductive biology in these hybrids would help explain the origin and persistence of these divergent nuclear alleles. For instance, if the hybrids cannot reproduce sexually, as has been documented for *B. medranoana*, persistence of interspecific copies would suggest that it is a F1 hybrid. In the absence of meiosis, concerted evolution has been shown to proceed slowly (*Amelanchier*, Campbell et al., 1997) although gene conversion (Hillis et al., 1991) can still occur.

Phylogenetic incongruence of *Bursera ovata* between nuclear and chloroplast phylogenies indicates a more complicated evolutionary history for this species. In nuclear phylogenies, all clones of nuclear markers for *B. ovata* nested with those of *B. spinescens*, whereas in chloroplast phylogenies *B. ovata* either nested in resolved clades or polytomies with *B. simaruba*. The existence of unobserved *B. simaruba* nuclear alleles in *B. ovata* is improbable based on the fact that cloning studies of two nuclear markers failed to isolate them and the fact that both nuclear markers can be sequenced directly without cloning. Lineage sorting may be responsible for the loss of ancestral polymorphic nuclear alleles although this phenomenon is not observed among the multiple copies of ETS present in nonhybrid *Bursera* taxa (Fig. 9). If *B. ovata* is derived from hybridization, it would represent *B. simaruba* (♀) × *B. spinescens* (♂), which is the reciprocal of *B. gracilipes* (= *B. spinescens* × *B. simaruba*). However, the lack of nuclear markers from *B. simaruba* in *B. ovata* does not confirm whether *B. ovata* is a stabilized hybrid taxon, a backcross generation, or merely a well-homogenized F1.

One of the hypothesized mechanisms of concerted evolution is unequal crossing over during meiosis, which reduces and

eventually eliminates heterogeneous alleles of high-copy gene regions in the gamete haploid genome (Hillis and Dixon, 1991). Complete elimination of heterogeneous copies either by uneven crossing over or by gene conversion can require multiple backcross generations (*Armeria*, Fuertes Aguilar et al., 1999) or may happen immediately during F1 formation (*Zea*, Zimmer et al., 1988). Repeated backcrossing of *B. simaruba* × *B. spinescens* (as the maternal parent) to *B. spinescens* (as the paternal parent) could also reduce or eliminate the number of maternally inherited copies of nuclear alleles from *B. simaruba* within the hybrid lineage. In this sense, *B. ovata* could represent the introgression of *B. simaruba* cytoplasm into a *B. spinescens* nuclear genetic background. Given that sterility barriers between species of Hispaniolan *Bursera* appear to be low, the backcross scenario may be a more probable explanation for the phylogenetic incongruence of *B. ovata* than the other alternatives discussed.

Additional tests of the reproductive biology of all Hispaniolan *Bursera* species and finer-scale genetic investigations of multiple accessions of the hybrids are needed to determine whether these hybrids are first generation hybrids, backcross generations, or stabilized species. Trees identified as *B. ovata*, *B. brunea*, and *B. gracilipes* have been collected from multiple localities within low-elevation, arid, karstic habitats of Barahona and Pedernales Provinces in the southeastern portion of the Dominican Republic as well as from Haiti (except *B. ovata*). None of the hybrids may represent a stable species; instead, each may be an assemblage of independently derived hybrids or backcross generations. The long life cycle of *Bursera* precludes breeding experiments that might investigate the long-term genetic fate of these interspecific hybrids. In one study of population dynamics of *Bursera* in the Galápagos, individual trees were estimated to have a 200-year lifespan (Hamann, 2001) although no published data about the reproductive phenology of *Bursera* exist. Natural hybrid zones, such as the region in southwestern Dominican Republic, offer a chance to observe multiple hybrid generations with varying genetic backgrounds simultaneously. Their value is tempered by the fact that detrimental epistatic interactions between genomes (Rieseberg and Buerkle, 2002; Levin, 2003) and other unfit hybrid combinations are less likely to be observed due to natural selection.

Of the 17 *Bursera* species distributed in the Caribbean, 15

belong to *B.* subgenus *Bursera* and 15 are endemic to this region (Table 2). These endemic species include *B. inaguensis*, *B. spinescens*, and *B. ovata*, which have been transferred to *Commiphora* (Moncada-Ferrera, 1989; Borhidi, 1992) but are clearly part of *B.* subg. *Bursera* based on our phylogenetic results. The long history of *Bursera* collection in the Caribbean may have resulted in redundant species descriptions and, as a result, precise enumeration of all Caribbean *Bursera* species will require a taxonomic revision. Interestingly, our phylogenetic analyses suggest that nonhybrid *Bursera* species endemic to the Caribbean share a common ancestor (Fig. 11). The need for a revision of Caribbean *Bursera* is highlighted further by results of this study that indicate interspecific hybridization can complicate species identification (e.g., *B. brunea*, *B. gracilipes*, and *B. ovata*). It is possible that other described species of Caribbean *Bursera* may be interspecific hybrids that involve the widespread species *B. simaruba* and the island endemic species.

Historically, hybridization among *Bursera* species has been a well-accepted phenomenon but the long-term effects of these hybrids on the evolution of the genus have been a matter of some speculation. Three different cases of hybridization within *Bursera* now have been confirmed using molecular genetic techniques. It appears that the genetic composition and the long-term fate of hybrid entities may vary. The Mexican hybrid, *Bursera medranoana*, reproduces asexually (Cortés-Palomec, 1998). The two interspecific hybrids collected in the Dominican Republic, *B. brunea* and *B. gracilipes*, may be sterile like *B. medranoana* but await further experimental field tests. *Bursera ovata* may be a sexually reproducing, stabilized hybrid taxon or a backcross generation. In either scenario, the sexual reproduction of *B. ovata* could have significant implications for interpreting the evolutionary history of *Bursera*. Cross-breeding experiments of *Bursera* species and genetic tests of seed progeny should reveal the ease with which hybrid combinations can form.

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